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Metal and Radioactive Elements Uptake of Wild *Agaricus* and *Agrocybe* Species Growing in Samanlı Mountains (Türkiye)

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Abstract: Twenty-two element contents were analyzed by ICP-AES equipment in five wild *Agaricus* and *Agrocybe* taxa [*Agaricus bresadolanus* Bohus, *A. sylvicola* (Vittad.) Peck, *A. xanthodermus* Genev., *Agrocybe paludosa* (J.E. Lange) Kühner & Romagn. ex Bon and *Agrocybe praecox* (Pers.) Fayod] from Samanlı Mountains of Türkiye. The element uptake was observed at the different levels in each *Agaricus* and *Agrocybe* species. The highest Pb and P concentrations were determined as 16.74 mg/kg and 1.501 mg/kg in *A. sylvicola* and *A. bresadolanus* respectively. Ag, Hg, and P concentrations were determined as 30685 µg/kg, 59781 µg/kg, and 501 mg/kg in A. bresadolanus respectively. *A. sylvicola* has the highest Ni, Cu, and Mn concentrations as 37.1, 43.63 and 1476 mg/kg respectively, whereas *A. praecox* has the highest Zn, Cd, and Ba concentrations of 336.8, 2.26, and 571.5 mg/kg. The highest K concentration was found in A. xanthodermus with 5.31 mg/kg.

According to WHO and FAO criteria, there is no important risk for the element uptake for human health if these species would be consumed. Additionally, some radioactive metals were found in mushroom species such as Sr, V, Th, Sc, Ga, and U. People should be careful against radioactive pollution if they consume mushrooms naturally.

Key words: Agaricus, Agrocybe, ICP-AES, element uptake, radioactivity, Türkiye.

Samanlı Dağları'nda (Türkiye) Yetişen *Agaricus* ve *Agrocybe* Türlerinin Metal ve Radyoaktif Element Alımı

Öz: Türkiye'nin Samanlı Dağlarında yetişen beş doğal *Agaricus* ve *Agrocybe* taksonunda [*Agaricus bresadolanus* Bohus, *A. sylvicola* (Vittad.) Peck, *A. xanthodermus* Genev., *Agrocybe paludosa* (J.E. Lange) Kühner & Romagn. ex Bon ve *Agrocybe praecox* (Pers.) Fayod] ICP-AES metodu ile yirmi iki element içeriği analiz edilmiştir. *Agaricus* ve *Agrocybe* türlerinin her birinde farklı seviyelerde element alımı gözlemlendi. En yüksek Pb ve P konsantrasyonları sırasıyla *A. sylvicola* ve *A. bresadolanus*'ta 16.74 mg/kg ve 1.501 mg/kg olarak belirlendi. *A. bresadolanus*'ta Ag, Hg ve P konsantrasyonları sırasıyla 30685 µg/kg, 59781 µg/kg ve 501 mg/kg olarak belirlendi. *A. sylvicola* sırasıyla 37.1, 43.63 ve 1476 mg/kg ile en yüksek Ni, Cu ve Mn konsantrasyonlarına sahipken, *A. praecox* 0.54 mg/kg, 10.20 mg/kg ve %27.9 ile en yüksek Mo, Ni ve P konsantrasyonlarına sahiptir. *A. paludosa* 336.8, 2.26 ve 571.5 mg/kg ile en yüksek Zn, Cd ve Ba konsantrasyonlarına sahiptir. En yüksek K konsantrasyonu 5.31 mg/kg ile *A. xanthodermus*'da bulunmuştur.

WHO ve FAO kriterlerine göre bu türlerin tüketilmesi durumunda element alımı için insan sağlığı açısından önemli bir risk bulunmamaktadır. Ayrıca Sr, V, Th, Sc, Ga ve U gibi bazı radyoaktif metaller mantar türlerinde bulunmuştur. İnsanlar, mantarları doğal yollarla tüketirken radyoaktif kirliliğe karşı dikkatli olmalıdır.

Anahtar kelimeler: Agaricus, Agrocybe, ICP-AES, element alımı, radyoaktivite, Türkiye.



Introduction

People have used macrofungi as food or treatments for some diseases for centuries. They can grow spontaneously in nature, and some species can be cultured. Naturally, grown species can be classified as poisonous, edible, or inedible. Toxins of the poisonous mushrooms can damage the intestine, the lungs, and the central nervous system (Seeger and Stijve, 1980). Edible macrofungi species have important vitamins and mineral substances for human health. While 92 % of the fresh mushrooms have water, 8 % of the remaining portion of them contains protein, fat, carbohydrate, vitamins, calcium, phosphorus, potassium, iron, copper, fiber, ash, etc (Matilla et al., 2002).

Macrofungal species are also a rich mineral resource. Especially, mushrooms can accumulate some essential minerals, and they can be beneficial for people with mineral deficiency. Minerals are grouped as macro (calcium, phosphorus, potassium, sulfur, chlorine, sodium, and magnesium) and micro minerals (iron, manganese. cobalt, copper, zinc, molybdenum, vanadium, chromium, tin, fluorine, silicon, selenium, and iodine). Micro mineral amounts in living organisms are extremely low due to the presence of trace elements. Despite iron, manganese, cobalt, copper, zinc. molybdenum, vanadium, chromium and tin are metals fluorine, silicon, selenium, and iodine are non-metals. Elements that have been classified as essential, beneficial, or detrimental are necessary for the survival and health of animals and humans (Doğan et al., 2006). Mushrooms are valuable health diets and have low in calories, and high in vegetable proteins, iron, zinc, chitin, fiber, vitamins, and minerals (Demirbaş, 2001). The contents of mushrooms range from crude protein 3.6-39.9 (% DM), crude fat content 0.5-6.3 (% DM), and carbohydrate 39-91 (% DM) (Vetter, 2019). Numerous data on the contents of major elements, and especially trace elements have been available in the literature (Kalač 2010; Kalač, 2012).

Wild-growing mushroom consumption is preferred over cultivated species in many central and Eastern Europe countries. Collection of the mushroom in nature has recently become a highly valued recreational activity in these countries as a lasting part of cultural heritage (Kalač, 2010). Mushrooms have also been picked up in Türkiye's forests [especially *Fagus orientalis* Lipsky. (Doğu kayını), *Carpinus betulus* L. (Gürgen), *Castanea* sativa Mill. (Kestane), Abies spp. (Göknar), Quercus spp. (Meşe), and Pinus spp. (Çam) forests from Marmara Region]. When collecting mushrooms from nature, attention should be paid to the metal contents and the mushrooms collected from contaminated areas should not be consumed. Some authors have reviewed the literature about the heavy metal concentration in mushrooms and have presented few data about the metal concentration in mushrooms from Agaricus genera (Tüzen et al., 1998; Michelot et al., 1998; Kalać and Svoboda, 2000; Cocchia et al., 2006; Tüzen et al., 2007; Melgar et al., 2009) and Agrocybe (Tüzen et al., 1998; Michelot et al., 1998; Kalać and Svoboda, 2000; Melgar et al., 2009). Although naturally grown mushrooms are consumed a lot in the research area, there is no study on the element contents. The main purpose of this study is to determine the harmful or beneficial aspects of some of the mushrooms in the region by doing an element analysis.

Material and Metod

Mushrooms were collected from the Samanli Mountains, which are located on the northwest side of Türkiye. To identify the samples, the habitat and morphological characteristics of the mushrooms in the localities were noted and photographed. The spore prints of mushroom samples were obtained and spore measurements were determined in the laboratory. Some reagents such as Melzer's reagent, 5% KOH, HNO3, Aniline, etc were used as chemical substances. Samples were identified by using reference books (Moser 1983, Breitenbach & Kränzlin 2000). A voucher sample for each species is kept at Selcuk University, Mushroom Application and Research Centre, Konya/Türkiye.

Identified samples were cleaned, and cut into slices, and the samples were washed with deionized water. Each sample was dried at 50°C overnight and crushed in a mortar and pestle. Digestion of mushroom samples was performed using an oxi-acidic mixture of HNO₃: H₂SO4: H₂O₂ (4: 1 : 1: 12 mL for 2 to 4-g sample) and heating at 75°C for 3 h. After cooling, 20 mL of deionized water was added and the digest was again heated up to 150°C for 4 h and brought to a volume of 25 mL with deionized water. The metal content of the mushroom samples was determined by ICP-AES (Varian Vista Ax Model). The equipment automatically yielded triplicates for each sample, averaged the data, and calculated the relative standard deviations.

Localities, habitat, and collection numbers of the species are given in Figure 1 and Table 1.

In addition, the Turkish names of the species are given in Table 1 according to Sesli et al. (2020).

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Figure 1. The localities in the study area.

Table 1. The	e localities an	nd habitats of	f the mushroom sar	nples.

Locality No	Taxa and Turkish			Location	Habitat	Coordinate	Altitude
93		Sakarya	Geyve	Şimşirlik boğazı	Abies nordmanniana subsp. bornmuelleriana and Buxus sempervirens forest	40°30'55N- 30°33'54E	1250m
120	<i>A. sylvicola</i> (Boylu Kızıl)	Sakarya	Geyve	Şimşirlik boğazı		40°30'55N- 30°33'54E	1250m
22	A. bresadolanus (Halkalıkızıl)	Sakarya	Akyazı	Soğuksu		40°39'06N- 030°43'37E	930m
117	A. xanthodermus (Ağulu kızıl)	Kocaeli	Yuvacık	İnönü plateau		40°34'12N- 030°00'17E	1065m
107	<i>A. paludosa</i> (Yaş metelik)	Sakarya	Geyve	Karagöl plateau, Mahdumlar village	pornmuelleriana, Carpinus orientalis, F.	40°30'17N- 030°34'39E	1150m
26	<i>A. paludosa</i> (Yaş metelik)		Geyve	Karagöl plateau, Mahdumlar village	A. nordmanniana subsp. bornmuelleriana, C. orientalis, F. orientalis, B. sempervirens forest	40°30'17N- 030°34'39E	1150m
88	<i>A. paludosa</i> (Yaş metelik)	Sakarya	Geyve	Acıelma	E orientalis (' orientalis torest	40°35'49N- 030°10'60E	1060m
161	<i>A. praecox</i> (Bahar meteliği)	Kocaeli	Suadiye	Altioluk plateau, Kuzuyayla, in national park	E orientalis torest	40°37'28N- 030°07'06E	1325m
33	<i>A. praecox</i> (Bahar meteliği)	Sakarya	Akyazı	Dokurcun, Dikmentepe	A. nordmanniana subsp. bornmuelleriana forest	40°39'03N- 030°53'28E	1350m

Results

Element concentrations of the species (*Agaricus* bresadolanus, *A. sylvicola*, *A. xanthodermus*, *Agrocybe* paludosa, and *A. praecox*) are given in Table 2.



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Table 2. The element content of mushroom taxa in the research area (n=3, S.D. was not presented in the table											
		mg/kg	mg/kg	mg/kg	%	mg/kg	µg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Locality											
No	Таха	Cd	Pb	Ni	Fe	Zn	Ag	Со	Cu	Мо	As
93	A. sylvicola	0.23	3.73	37.1	0.165	171.8	298	0.87	13.54	0.33	1.3
120	A.sylvicola	1.63	16.74	20.1	1.034	174.5	31	7.49	43.63	0.31	5.0
	Α.										
22	bresadolanus	0.45	1.26	10.2	0.205	90.5	30685	1.03	41.65	0.25	3.2
	Α.										
117	xanthodermus	0.18	2.08	3.1	0.046	47.7	95	0.31	5.08	0.25	0.4
107	A. paludosa	1.41	1.18	8.4	0.090	336.8	53	0.43	17.51	0.11	405.4
26	A. paludosa	0.22	0.81	4.3	0.025	68.4	2838	0.25	19.30	0.13	6.4
88	A. paludosa	2.26	1.08	27.3	0.223	73.9	27868	1.93	72.31	0.12	0.5
161	A.praecox	1.27	12.40	10.5	1.319	61.9	236	10.20	32.67	0.54	3.9
33	A. praecox	0.18	36.61	1.80	58.8	68	21.1	0.87	338	0.153	1.6

		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	%	mg/kg
Locality No	Таха	Th	Sr	Sb	Bi	Mn	к	S	Са	Р	v
93	A. sylvicola	0.19	11.7	0.09	0.03	77	2.27	0.12	0.34	0.707	3
120	A.sylvicola	1.17	12.5	0.36	0.24	1476	3.22	0.12	0.47	0.311	22
22	A. bresadolanus	0.04	2.1	0.03	<0.02	135	5.14	0.36	0.06	1.501	4
117	A. xanthodermus	0.11	2.1	0.03	<0.02	163	5.31	0.08	0.05	0.490	3
107	A. paludosa	0.16	2.8	<0.02	0.03	50	3.11	0.18	0.12	0.403	<2
26	A. paludosa	0.02	1.9	< 0.02	< 0.02	67	2.74	0.14	0.07	0.430	<2
88	A. paludosa	0.05	9.4	0.04	< 0.02	126	4.10	0.45	0.11	0.594	7
161	A.praecox	2.20	7.3	0.27	0.09	889	2.21	0.08	0.18	0.325	28
33	A. praecox	0.17	5.2	0.17	< 0.02	-	2.74	0.26	0.17	0.745	<2

		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	mg/kg	µg/kg
Locality											
No	Таха	La	Cr	Mg	Ba	Ti	В	AI	Na	U	Au
93	A. sylvicola	0.76	4.3	0.104	25.7	47	8	0.10	0.004	0.25	<0.2
120	A.sylvicola	10.01	19.1	0.282	91.9	120	3	1.02	0.004	0.20	4.0
	Α.										
22	bresadolanus	0.28	2.4	0.174	6.0	23	2	0.19	0.026	0.02	3.0
	Α.										
117	xanthodermus	0.31	6.1	0.138	7.3	19	4	0.05	0.006	0.06	<0.2
107	A. paludosa	0.56	2.5	0.093	30.1	17	2	0.07	0.007	0.02	<0.2
26	A. paludosa	0.10	3.2	0.092	17.0	3	4	0.02	0.014	<0.01	2.0
88	A. paludosa	0.25	6.7	0.139	571.5	95	<1	0.13	0.002	0.01	0.2
161	A.praecox	5.41	27.9	0.130	43.0	115	3	0.93	0.003	0.47	1.8
33	A. praecox	0.45	6.0	0.163	46.4	8	5	0.09	0.011	0.02	0.3

		mg/kg	mg/kg	mg/kg	µg/kg	mg/kg	mg/kg	mg/kg
Locality								
No	Таха	W	Sc	ТІ	Hg	Se	Те	Ga
93	A. sylvicola	0.1	0.4	0.04	64	0.4	<0.02	0.4
120	A.sylvicola	<0.1	2.6	0.11	70	0.3	0.14	2.2
	А.							
22	bresadolanus	<0.1	0.4	0.08	5978	7.5	<0.02	0.5
	Α.							
117	xanthodermus	<0.1	0.3	0.34	197	0.1	0.06	0.2
107	A. paludosa	<0.1	0.3	0.03	28	0.2	<0.02	0.2
26	A. paludosa	<0.1	0.2	<0.02	778	4.7	<0.02	<0.1
88	A. paludosa	<0.1	0.8	<0.02	519	0.8	<0.02	0.4
161	A.praecox	0.1	2.7	0.10	626	1.1	<0.02	2.8
33	A. praecox	<0.1	0.3	<0.02	128	0.1	<0.02	0.3

Discussions

According to Table 2, the highest element concentrations (14 different elements) was observed on *Agaricus sylvicola*, these elements are Ni with 37.1 mg/kg, Cu with 43.63 mg/kg, Sr with 12.5 mg/kg, Sb with

0.36 mg/kg, Bi with 0.24 mg/kg, M with 1476 mg/kg, Ca with 0.47 %, La with 10.01 mg/kg, Mg with 0.282 mg/kg, Ti with 120 mg/kg, B with 8 mg/kg, Al with 1.02 %, Au with 4 μ g/kg and Te with 0.14 Mg/kg. Second is *Agrocybe praecox* with 10 elements, they are Pb with 36.61 mg/kg,



Fe with 1.319 %, Co with 10.20 mg/kg, Mo with 0.54 mg/kg, Th with 2.20 mg/kg, V with 28 mg/kg, Cr with 27.9 mg/kg, U with 0.47 mg/kg, Sc with 2.7 mg/kg, Ga with 2.8 mg/kg. The third are *Agaricus bresadolianus* and *Agrocybe paludosa* with 5 elements for each. These elements are Ag with 30685 μ g/kg, P with 1.501%, Na with 0.026%, Hg with 5978 μ g/kg, Se with 7.5 mg/kg for *Agaricus bresadolianus*; Cd with 2.26 mg/kg, Zn with 336.8 mg/kg, As with 405.4 mg/kg, S with 0.45 %, Ba with 571.5 mg/kg for *Agarocybe paludosa*. Last, *Agaricus xanthodermus* has the two highest elements; they are K with 5.31 mg/kg and TI with 0.34 mg/kg.

The highest Ni was observed as 37.1 mg/kg in A. sylvicola. There is evidence that nickel is an essential trace element in several animal species, plants, and prokaryotic organisms. Nickel appears to be essential for humans, although no data are available concerning nickel deficiency. Allergic skin reactions are the most common health effect of nickel, affecting about 2% of the male and 11% of the female population. Nickel content in consumer products, possibly in food and water is critical for the dermatological effect. The respiratory tract is also a target organ for allergic manifestations of occupational nickel exposure. Work-related exposure in the nickel-refining industry has been documented to cause an increased risk of lung and nasal cancers. Inhalation of a mixture of oxidic, sulfidic, and soluble nickel compounds at higher than 0.5 mg/m³ concentrations, which is often considerably higher, for many years has been reported. (Anonymous, 1990).

Foods normally are the major source of Cd intake. Available data indicate that the current intake of cadmium from the foods is most commonly 10-35 µg/d (WHO, 2000). The bioavailability of Cd can be markedly affected by nutritional factors. Low iron status, as determined in serum ferritin levels that are prevalent among women, increases the uptake of cadmium from the gastrointestinal tract. Besides, if Cd binds to phytates, metallothionein, and other proteins, the bioavailability of Cd from some grains, seeds, and foods may be reduced. Usually, people cannot get enough cadmium from water and food sources. Although water is not a major contributor to Cd intake for most individuals, elevated natural Cd levels in water can occur, and resultant Cd intakes can be as high as the dietary contribution. Estimates of mean Cd intake from national food surveys and total diet studies generally ranged from 0.1 to 0.5 µg/kg body weight per day. The estimates derived from the WHO regional diets, based on food balance sheets, range from 0.35 to 0.63 µg/kg body weight per day (WHO, 2000). Estimates of Cd intake (µg/kg body weight per day, assuming a body weight of 60 kg) in the WHO GEMS/Food regional diets for mushrooms is 0.001 (in Europe) and 0 (Middle East, Far East, Africa, and Latin America) (WHO, 2000). The WHO mentions maximum permissible levels in raw plant materials for cadmium as 0.30 mg/kg. The Cd

concentrations were observed as 2.26 mg/kg in *Agrocybe paludosa*. This is higher than limit values when we compare with WHO data. Nevertheless, there are some similar data for the macrofungi; the highest Cd concentration among *Agrocybe* taxa was reported as 3.35 mg/kg in *A. praecox* (Michelot et al., 1998). Among *Agaricus* taxa, the highest Cd value was reported in *A. bresadolianus* (Michelot et al., 1998).

Pb has no beneficial role in human metabolism, producing progressive toxicity. Pb is the most toxic heavy metal, and the inorganic forms are absorbed through ingestion by water and food, and inhalation. An especially serious effect of lead toxicity is its teratogenic effect. Pb poisoning also causes inhibition of the synthesis of hemoglobin dysfunctions in the kidnevs, joints. reproductive system, cardiovascular system, and chronic damage to the nervous system (Duruibe et al., 2007). The EU maximum permitted level for lead in cultivated mushrooms is 0.3 mg/kg wet weight (European Commission, 2001). Pb was determined as 36.31 mg/kg in A. praecox and this level is high according to the EU level.

Iron is the most commonly used element with 4.2% in plants, animals, and people after aluminum. Although it is normally in an insoluble form, it can be a soluble form of iron by many natural reactions and contaminate groundwater (Gray, 1996). The tolerable value is 50-100 mg/kg daily amount in normal people. The lethal dose for an adult human is 100 grams (WHO-FAO, 1996). Fe was determined from some mushrooms in Türkiye by different researchers and it was found as; 341.98±2.58 mg/kg in Tricholoma terreum, 84.51 ±5.73 mg/kg in Coprinus micaceus, 451.3±4.8 mg/kg in cultured Pleurotus ostreatus and 57.1±1.1 mg/kg in naturel P. ostreatus, and 264.57 ±17.27 mg/kg in Morchella esculenta (Akgül et al., 2016; Sevindik et al., 2016; Eraslan et al., 2021). There is no risk in terms of the highest iron content (1.319%) determined in A. praecox.

90% of zinc in the soil is stored in plants. Zinc is an essential mineral for the organism. Zinc has roles in carbohydrate, protein, lipid, nucleic acid, HEM synthesis, expression, reproduction, growth, gene and embryogenesis. It also plays a critical role in the structural and functional integrity of the cells (Belgemen and Akar, 2004). The maximum daily dose in the US is 15 mg/kg for adult men and 12 mg/kg for women (WHO-FAO, 1996). The Zn in A. bresadolianus was determined as 336.8 mg/kg and this amount is higher than the literature. Similar results have been obtained in previous studies. These are 107.11±7.82 mg/kg in Tricholoma terreum, 51.01±7.42 mg/kg in Coprinus micaceus, 134.9±2.1 mg/kg in cultured Pleurotus ostreatus and 45.9±0.9 mg/kg in naturel P. ostreatus (Akgül et al., 2016; Sevindik et al., 2016). Nevertheless, Zn was found in more lees than the other species with a value of 7.82 ±0.34 in Morchella esculenta (Eraslan et al., 2021).



According to WHO, EU standards, and the regulation issued by the Ministry of Health in 2005, the drinking water Hg limit is 1 ppb (μ /l). Airborne particles exceeding 10 mg/m³ are dangerous for health. Chemical pneumonia may occur at concentrations above 1 mg/m³. The maximum amount of mercury that can be found in foods was determined as 0.05 mg/kg by FAO/WHO. The weekly tolerable amount (PTWI) according to WHO standard is 0.0016 mg/kg (WHO-FAO 1996). The highest mercury value in *A. paludosa* is 5978 µg/kg and there is no health risk.

Although the average Al content in healthy human tissues in the UK is less than 0.5 μ g/g, it is observed as high as 2.6 μ g/g in the liver, 18.2 μ g/g in the lung, 32.5 μ g/g in the lymph nodes and 73.4 μ g/g in the bones (WHO 1989; WHO-FAO, 1996). Naturally, occurring aluminum, as well as aluminum salts used as coagulants in drinking water treatment, are the primary sources of aluminum in drinking water. The presence of aluminum at concentrations over 0.1–0.2 mg/L often leads to consumer complaints because of the deposition of aluminum hydroxide floc and the exacerbation of discoloration of water by iron (WHO 2017). Al was determined as 1.02 % in *A. sylvicola*, this level is tolerable.

Besides, some radioactive metals such as Sr, V, Th, Sc, Ga, and U were observed in mushroom species. Overall, radioactive uptake of *A. praecox* is higher than other species with the rates of 28 mg/kg for V, 2.20 mg/kg

for Th, 2.7 mg/kg, for Sc, 2.8 mg/kg for Ga, and 0.47 mg/kg for U. Sr (12.5 mg/kg) was only found more in Agaricus sylvicola. Radionuclides are naturally present in the environment. They may also enter the environment because of human activities. Natural sources of radiation are responsible for the large majority of radiation exposure (greater than 98%), excluding medical exposure. Additional exposure can result from human activities associated with radioactive materials (Health Canada, 2009). Guidance levels for common natural and artificial radionuclides are 10 Bq/l (Uranium-238), 1 Bq/l (Uranium-234), 1 Bq/I (Thorium-230). The provisional guideline value for the total content of uranium in drinking water is 30 µg/L based on its chemical toxicity, which is predominant compared with its radiological toxicity (WHO, 2017). Samanlı Mountains are very close to industrial areas in Kocaeli and Sakarya regions. Therefore, the risk of chemical, biological and radioactive contamination is very high in regions close to industrial zones.

People should be careful about radioactive and heavy metal pollution when they consume mushrooms naturally.

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